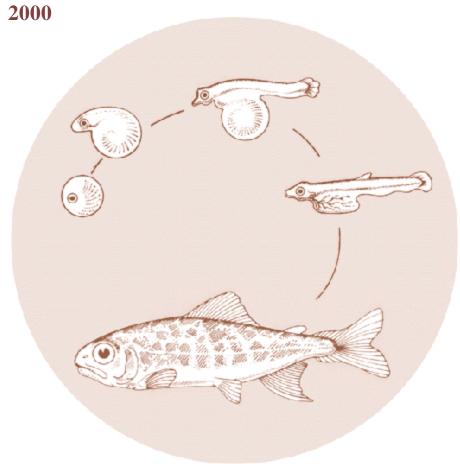
# Tucannon River Spring Chinook Salmon Captive Broodstock Program

# **Annual Report**





#### This Document should be cited as follows:

Bumgardner, Joseph, Michael Gallinat, "Tucannon River Spring Chinook Salmon Captive Broodstock Program", Project No. 2000-01901, 34 electronic pages, (BPA Report DOE/BP-00000074-1)

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This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the author's and do not necessarily represent the views of BPA.

# Tucannon River Spring Chinook Salmon Captive Broodstock Program

# **FY2000 Annual Report**

FY2000 Project Period October 1999 - April 2001

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Project Number 2000-019-00 Contract Number 00000074-00001

**June 2001** 

# **Acknowledgements**

The Tucannon River spring chinook salmon captive broodstock program is the result of efforts by many individuals within the Washington Department of Fish and Wildlife (WDFW) and from other agencies.

We thank Butch Harty, Lyons Ferry Hatchery (LFH) Complex Manager, for his coordination efforts and dealing directly with the facility modifications at LFH. We thank Hatchery Specialist IV's Doug Maxey and Bruce Walters for their cooperation with hatchery sampling, providing information regarding hatchery operations and hatchery records, and their input on evaluation and research activities. We also thank all additional hatchery personnel who provide the day-to-day care of the captive brood and for their assistance with spawning, sampling, and record keeping.

We thank Lynn Anderson and Julie Hooff of the Coded-Wire Tag (CWT) lab and tagging staff for their assistance in CWT verification and assisting in tagging of the captive brood fish. We thank Steve Roberts for providing information on any fish health issues that arise in the captive brood fish. Dan Witzack (Hurd Creek Hatchery) provided training for sorting of mature captive brood fish, and assisted with design and operational procedures for the program.

We thank the Nez Perce Tribe (NPT) and Confederated Tribes of the Umatilla Indian Reservation (CTUIR) who participated and provided comments during the development of the Tucannon Master Plan. The NPT and CTUIR are also active members in the Broodstock Working Group, a technical advisory group among the co-managers to discuss issues that may arise during the program. We also thank the National Marine Fisheries Service for approving the captive broodstock program through the ESA Section 10 permit process, and finding the program necessary for recovery/stabilization of the Tucannon River spring chinook stock. Without these respective agencies' support, this program would not have proceeded.

We thank the Lower Snake River Compensation Plan (LSRCP), in particular Ed Crateau and Dan Herrig, for their financial support of the captive broodstock program from 1997-1999. Without LSRCP support and financial assistance, this program and facility modifications at LFH would never have been finished when needed for the fish.

We thank the Bonneville Power Administration (BPA) for their financial support during FY2000 and FY2001. We thank Greg Baesler (BPA contract officer) for his support and assistance regarding budget manners.

We thank Mark Schuck, Glen Mendel, Butch Harty, Steve Roberts, and Bill Hopley of the WDFW, Brian Zimmermann (CTUIR), Jim Harbeck and other anonymous staff from the NPT Enterprise Field Office, and Carlin McCauley (NMFS-Manchester) for their critical reviews and helpful comments on a draft of this report.

## **Abstract**

This report summarizes the objectives, tasks, and accomplishments of the Tucannon River spring chinook captive brood program from program inception (1997) through April 2001.

The WDFW initiated a captive broodstock program in 1997. The overall goal of the Tucannon River captive broodstock program is for the short-term, and eventually long-term, rebuilding of the Tucannon River spring chinook salmon run, with the hope that natural production will eventually sustain itself. The project goal is to rear captive salmon to adults, spawn them, rear their progeny, and release approximately 150,000 smolts annually into the Tucannon River between 2003-2007. These smolt releases, in combination with the current hatchery supplementation program (132,000 smolts), and wild production, is expected to produce 600-700 returning adult spring chinook to the Tucannon River each year from 2005-2010.

The Master Plan, Environmental Assessment, and most facility modifications at LFH were completed for the Tucannon River spring chinook captive broodstock program during FY2000 and FY2001. DNA samples collected since 1997 have been sent to the WDFW genetics lab in Olympia for baseline DNA analysis. Results from the genetic analysis are not available at this time.

The captive broodstock program is planned to collect fish from five (1997-2001) brood years (BY). The captive broodstock program was initiated with 1997 BY juveniles, and the 2000 BY fish have been selected. As of April 30, 2001, WDFW has 172 BY 1997, 262 BY 1998, 407 BY 1999, and approximately 1,190 BY 2000 fish on hand at LFH.

Twelve of 13 mature 97 BY females were spawned in 2000. Total eggtake was 14,813. Mean fecundity was 1,298 eggs/female based on 11 fully spawned females. Egg survival to eye-up was 47.3%. This low survival was expected for three year old captive broodstock females. As of April 30, 2001, WDFW has 4,211 captive broodstock progeny on hand. These fish will be tagged with blank wire tag without fin clips and released as smolts from Curl Lake Acclimation Pond into the Tucannon River during April 2002.

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# Introduction

## **Reporting Period**

This report summarizes the objectives, tasks, and accomplishments of the Tucannon River spring chinook salmon (*Oncorhynchus tshawytscha*) captive brood program through April 2001. This report, while originally intended to cover activities accomplished exclusively under the Fiscal Year (FY) 2000 contract, includes events and results obtained prior to Bonneville Power Administration (BPA) funding and during FY2001 activities as well. This was done to provide readers with the program rational and more complete results from the tagging, rearing, and spawning activities that have occurred since program inception.

# **Tucannon River Spring Chinook Program Overview**

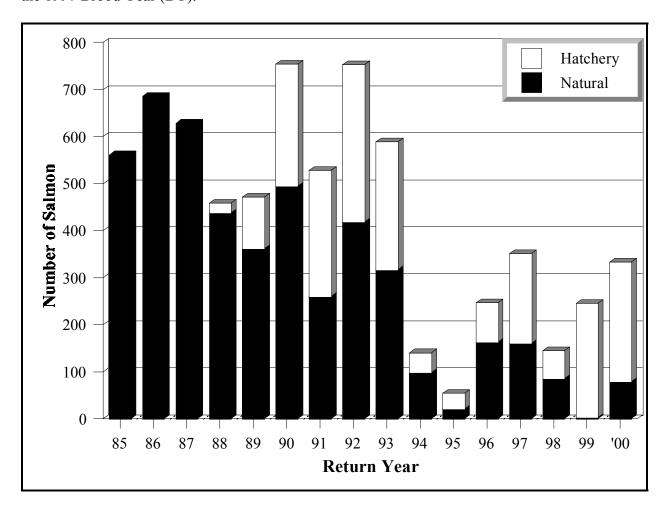
Prior to 1985, artificial production of spring chinook in the Tucannon River was nearly nonexistent, with only two fry releases (about 25,000 each) in two separate years in the 1960's. Neither of these releases is believed to have returned any significant number of adults. After completion of the four lower Snake River dams, the Lower Snake River Compensation Plan (LSRCP) program was formed to provide hatchery mitigation for spring chinook, fall chinook, and summer steelhead in the Snake River (USACE, 1975). In 1985, WDFW began the hatchery spring chinook production program in the Tucannon River by trapping wild (unmarked) adults for the hatchery broodstock. Hatchery-origin fish have been returning to the Tucannon River since 1988. The hatchery broodstock since 1989 has consisted of natural and hatchery-origin fish.

In 1992, the National Marine Fisheries Service (NMFS) listed Snake River spring/summer chinook as "endangered" (April 22, 1992 Federal Register, Vol 57, No. 78, p 14653), which included the Tucannon River stock. The listing status was changed to "threatened" in 1995 (April 17, 1995 Federal Register, Vol 60, No 73, p 19342). Between 1993-1998, WDFW operated the supplementation program under Section 10 direct take permit #848 for artificial propagation and research. Since 1998, WDFW has operated both the supplementation and captive broodstock program under Section 10 direct take permits #1126 (artificial propagation), and #1129 (research). The Endangered Species Act (ESA) allows for "the use of all methods and procedures which are necessary to bring any endangered species or threatened species to the point at which the measures pursuant to the Act are no longer necessary" (ESA 1973).

Consistent with that provision, WDFW and the co-managers decided in 1997 to implement this captive broodstock program to sustain and potentially recover this listed population. Both of the hatchery programs (supplementation and captive brood) are being conducted with the recognition that artificial propagation may have potentially deleterious direct and indirect effects on the listed fish (Hard et al. 1992; Cuenco et al. 1993; Busack and Currens 1995; Campton 1995). These effects may include genetic and ecological hazards that cause maladaptive genetic, physiological, or behavioral changes in donor or target populations, with attendant losses in natural productivity (Hard et al. 1992). However, WDFW and the co-managers believe the risk of extinction in the Tucannon River is high enough that aggressive intervention beyond the

current supplementation program is warranted. Further, this program has been defined to last for only one generation cycle (five years), and negative effects should be lessened due to the short term nature of the program.

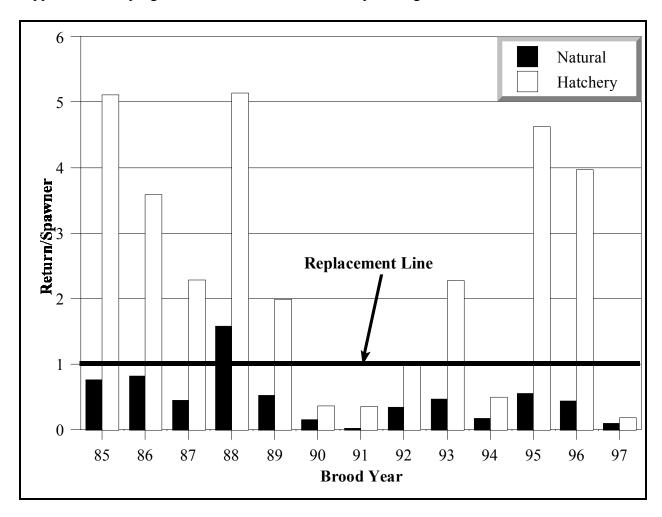
Adult returns between 1985-1993 were between 400-750 wild and hatchery fish combined (Figure 1). In 1994, the adult escapement declined severely to less than 150 fish, and the run in 1995 was estimated at 54 fish. In 1995, WDFW started the Captive Broodstock Program on their own but discontinued it based upon the 1996-97 predicted return estimates. Unfortunately, the 1996 and 1997 returns were not as strong as predicted. In addition major floods in 1996 and 1997 on the Tucannon River eliminated most natural production. Moreover, an 80% loss of the total hatchery eggtake occurred in 1997 due to an operation malfunction of a water chiller that cold shocked the eggs. Because of the lower returns, and losses to both natural and hatchery production, the Tucannon River spring chinook captive broodstock program was reinitiated with the 1997 Brood Year (BY).



**Figure 1.** Total estimated escapement for Tucannon River spring chinook salmon from 1985-2000.

Key to the Tucannon River spring chinook restoration effort will be whether or not the natural population can ever return above the replacement level. Since 1985 WDFW has monitored and

estimated the success of the natural population for comparison to the hatchery program as part of the LSRCP program (LSRCP 1998). Monitoring efforts to date has shown the natural population below the replacement level almost every year (Figure 2). In short, unless the natural population returns to a point above the replacement level, then both the captive broodstock and supplementation program will fail to achieve their respective goals.

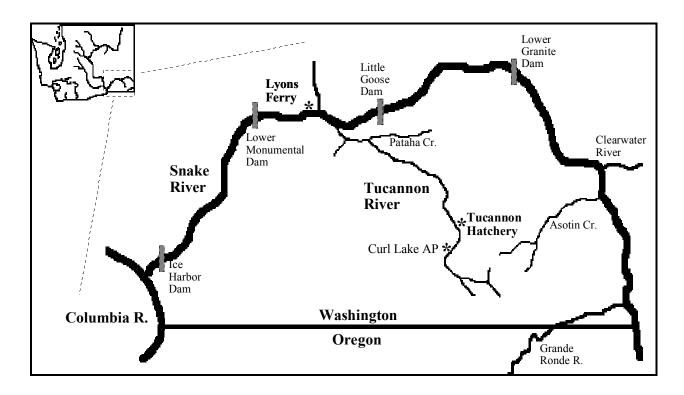


**Figure 2**. Return per spawner ratio (with replacement line) for Tucannon River spring chinook salmon for 1985-1997 brood years.

#### **Tucannon River Watershed Characteristics**

The Tucannon River empties into the Snake River between Little Goose and Lower Monumental dams approximately 622 river kilometers (rkm) from the mouth of the Columbia River (Figure 3). Stream elevation rises from 150 m at the mouth to 1,640 m at the headwater (Bumgarner et al. 2000). Total watershed area is about 1,295 km². Mean discharge is 174 cfs with a mean low of 61.5 cfs (August) and a mean high flow of 310 cfs (April/May). Local habitat problems related to logging, road building, recreation, and agriculture/livestock grazing has limited the production potential of spring chinook in the Tucannon River. Spring chinook typically spawn and rear above rkm 40. WDFW and the co-managers believe smolt releases in the upper

watershed have the best chances for high survival rates, and recovery effects from this program and the supplementation program will be maximized.



**Figure 3.** Location of the Tucannon River within the Snake River Basin, and locations of Lyons Ferry Hatchery, Tucannon Hatchery, and Curl Lake Acclimation Pond within the Tucannon River Basin.

It is hoped that recent initiatives for habitat improvement within the Tucannon Basin (BPA funded - Tucannon River Model Watershed Program, and the State of Washington Governor's Salmon Recovery Plan) which are aimed at increasing in-river survival, changing and improved ocean conditions, and continued adult and juvenile passage improvements at Federal Columbia River Power System (FCRPS) dams, will be enough to return the natural population above the replacement level. For example, broad based goals of the Model Watershed Program are to: 1) restore and maintain natural stream stability, 2) reduce water temperatures, 3) reduce upland erosion and sediment rates, and 4) improve and reestablish riparian vegetation. Managers hope that these in-river recovery efforts should ultimately increase survival of naturally reared spring chinook in the river. While this will only provide an increase to population numbers (parrs or smolts), greater numbers will return more fish back to the Tucannon River even if the passage problems and ocean conditions remain unchanged. The captive brood program should provide a quick increase in the number of adults that will produce progeny to take advantage of these habitat improvements.

# **Facility Descriptions**

The spring chinook supplementation program currently utilizes three different WDFW facilities: Lyons Ferry Hatchery (LFH), Tucannon Fish Hatchery (TFH), and Curl Lake Acclimation Pond (AP). Each of these facilities will also be used in some manner for the captive broodstock program for rearing, release and subsequent adult capture upon return. Lyons Ferry is located on the Snake River (rkm 90) at its confluence with the Palouse River (Figure 3). Lyons Ferry was constructed with funds provided by the Army Corps of Engineers, and has subsequently been funded through LSRCP program of the U.S. Fish and Wildlife Service. Ultimately, the FCRPS through BPA bears the cost of the LSRCP program. Lyons Ferry is used for adult broodstock holding and spawning, and incubation and early life rearing until production marking. Tucannon Hatchery, located at rkm 59 on the Tucannon River, has an adult collection trap on-site. Following marking at LFH, juveniles are transferred to TFH to rear through winter. In mid-February, the spring chinook are transferred to Curl Lake AP for a minimum of three weeks acclimation. Curl Lake AP is a 2.1 acre natural bottom lake with a mean depth of nine feet (pond volume estimated at 784,000 ft³). During the middle of March, the pond exit is opened and the fish are allowed to volitionally migrate from the lake until the third week of April.

Initially, WDFW proposed the use of two facilities to hold the captive broodstock program. In order to lessen the risk should disease outbreaks or facility water system failures occur at one of the facilities. For convenience of locality, we had initially proposed LFH and TFH for the program, with 65% of the fish to be reared at LFH, as LFH has more space and better water quality (cooler well water). The split was recommended for the maturing adults only, with produced progeny reared at LFH until transfer to TFH prior to release as smolts. However, due to extra facility modifications required at TFH, the initial set-up cost to the program was too high, and adequate funds could not be secured. The facilities needed to maintain the same program goals were then reallocated with the current plan to have all fish reared at LFH.

# **Spring Chinook Monitoring and Evaluation**

As previously mentioned, the LSRCP Tucannon River spring chinook supplementation program has an ongoing hatchery evaluation program. Some of the monitoring and evaluation activities include: smolt release sampling, smolt trapping, spawning ground surveys, genetic monitoring, snorkel surveys for juvenile population estimates, spawning, fecundity monitoring, and experimental release strategies for smolts. Through these and other activities, survival rates of the natural and hatchery fish have been documented for the span of the supplementation program. These same and other activities will continue to play a major role in evaluating the success of the captive broodstock program in the future (parent and progeny).

As part of the monitoring plan, survivals and rate of maturation are being documented by family groups within each brood year. Fecundity and egg size in relation to spawning success will be documented for all spawned captive broodstock females. Maturation timing will be monitored as well as overall growth rates for each brood year. Smolt migration will be monitored through the use of Passive Integrated Transponder (PIT) tags, and adult return rates will be monitored through adult trapping and carcass recoveries on spawning ground surveys.

# **Captive Broodstock Program**

The overall goal of the Tucannon River spring chinook salmon (*O. tshawytscha*) captive broodstock program is for the short-term, and eventually long-term, rebuilding of the natural run, with the hope that natural population will eventually sustain itself. The current hatchery mitigation goal under the LSRCP is to return 1,152 adult spring chinook of Tucannon River stock to the river annually. Attempts to reach the mitigation goal have been occurring through the current LSRCP supplementation program with the release of 132,000 smolts at 15 fish/lb (fpp), but have failed, largely because of poor smolt-to-adult survival rates. Currently, there is not an escapement goal for naturally produced spring chinook in the Tucannon River. It is hoped that through renegotiation of the Columbia River Fish Management Plan (CRFMP), an agreed upon goal will be established to better manage the population in the future.

The captive broodstock program is not intended to replace the current hatchery supplementation program. Rather, it is to provide a quick "boost" to the population in the short term because of poor runs initially predicted through 2001. A quick "boost" would not be possible under the existing supplementation program as it would require about 200 adults for the hatchery broodstock each year. This was not believed possible by the managers, as expected returns from 1998-2001 were less than 200 total fish annually. Further, that would mean taking more fish from the river, and nearly eliminating any natural production potential.

WDFW and co-managers believe that the low runs between 1997-2001 are expected to limit both the natural and hatchery production, possibly to a point where the run would not be able to fully recover. It is hoped that this program will delay or prevent extinction of the Tucannon River spring chinook salmon. However, this captive broodstock program and the supplementation program will not likely recover the population without a substantial increase in survival of spring chinook throughout the system. The specific objectives of the program are to rear spring chinook salmon to adults, spawn them, rear their progeny, and release the progeny as smolts into the Tucannon River. The program is scheduled to terminate with the final release of smolts in 2008. Successes and failures during and after the program ends will be evaluated by WDFW concurrently with the LSRCP hatchery evaluation program.

Eggs/fry to be incorporated into the captive broodstock program will only be collected from the 1997-2001 BYs from the supplementation program. The captive broodstock goal is to collect 290,000 eggs/year from captive brood females when an anticipated three complete age classes (Age 3-Age 5) are spawned concurrently. Under original program design, these eggs are expected to produce about 150,000 smolts for release from the Curl Lake AP. Depending on smolts produced each year this should provide a return of about 300 adult fish of captive broodstock origin per year between 2005-2010. These fish combined with fish from the hatchery supplementation program and natural production from the river should return 600-700 fish annually between 2005-2010. While this return is still well below the LSRCP mitigation goal, it increases the population level back to pre-1994 run sizes.

Captive brood program production (adults, eggs, or juveniles) in anticipated excess of the smolt goal may be released by other methods as discussed in the Master Plan (WDFW 1999). Options

include adult outplants, remote site incubation, fry outplants, or smolt releases into other systems (Asotin Creek).

The spring chinook captive broodstock program in the short term will help ensure that the Tucannon River spring chinook population is preserved until habitat-related factors and passage problems affecting the productivity and survival of wild fish can be remedied. The captive brood program, in conjunction with the supplementation program, is intended to facilitate recovery of the natural population, while minimizing the risk of further decline and restricting genetic changes which might result from artificial propagation. Monitoring and evaluation programs are in place to assess and adjust the effects of the captive broodstock program as needed (Bumgarner and Schuck 1999, Bumgarner et al. 2000). Measures have been taken to minimize and mitigate potential genetic and/or ecological hazards of this program to the listed population (WDFW 1999).

#### **Prior Actions and Results**

#### **Facility Modifications**

Fifteen 4 ft diameter circular starter tanks had been purchased when the captive broodstock program was started in 1995. In 1999, LSRCP purchased and supplied the needed funding for installation of eight 20 ft diameter circular rearing tanks for the adults, and for relocation of the small circular tanks. The fifteen 4 ft circular tanks and the eight 20 ft circular tanks were installed during August and September of 1999 in the captive broodstock rearing area at LFH. Construction followed the general outlay of the conceptual design (Appendix A).

#### **Source of Captive Population**

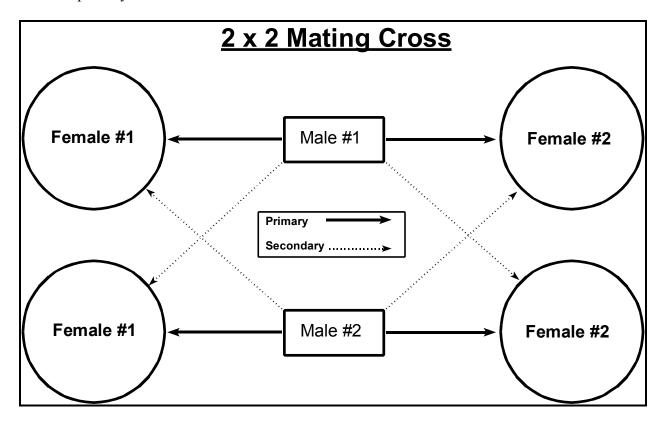
As described in detail in the Tucannon Master Plan (WDFW 1999), the captive population will come from the hatchery supplementation program during the 1997-2001 BYs. Supplementation broodstock consist of both natural and hatchery returns (generally 1:1 ratio). Returning hatchery fish used in the supplementation broodstock are verified to have come from the Tucannon River stock through Coded-Wire Tag (CWT) verification. Collection of eggs/fry from the supplementation program was done to lessen the effects of mining more fish from the natural population. Also, disease history and origin of parents would be known, and the overall effect to the supplementation program would be minimal.

Selection of eggs/fry is based on Bacterial Kidney Disease (BKD) and virology screening of females, and parent origin, and matings (Appendix B). Only females which are given a "Low" (0.11 - 0.19 Optical Density (OD)) or "Below Low" (< 0.11 OD) ELISA (BKD indicator) result are used for selection, with priority given to "Below Low" females. Priority for selection (in the following order) of eggs/fry is given to Wild x Wild, Wild x Hatchery (Mixed), and Hatchery x Hatchery crosses. All BYs identified for the program will follow the same criteria.

Screening for BKD was major factor in WDFW's decision to collect egg/fry from the supplementation program. By having the test results prior to selection, and by having rearing criteria that called for minimal sampling/handling, we felt our chances for breaking with BKD

would be minimized. To date, we know of no mortalities that can be attributed to BKD in the captive brood population

During the spawning process in the supplementation program, the eggs of two females are split in half with each lot fertilized by a different primary male (each male also acts as a secondary male). Due to the relatively small population size, this 2x2 mating (Figure 4) strategy has been incorporated into the supplementation program to increase genetic variation. Milt from a secondary male is added as a backup 30 seconds later. Actual fertilization takes place in a few seconds, so the backup male may not contribute equally to each individual egg lot unless semen from the primary male is non-viable.



**Figure 4.** Diagram of the 2x2 mating scheme used by WDFW in the supplementation and captive broodstock program.

However, because of the crosses, some progeny from the two females are likely related and considered a family unit. Therefore, we consider all crosses with identical males (whether as primary or secondary to the mating) as one family unit to avoid in-family matings in the future. So while only 15 "family" units are chosen for the program, actual contribution of male and female parents (population size) to the captive broodstock program on a yearly basis will be higher.

After the eggs have hatched and absorbed their yolk sac, they are ready to be placed in the rearing vessels and the selection process begins. Eighty fish (or generally 40 fry/female) from each of the 15 "family units" are selected (1,200 total fish) from each BY and moved to the 4 ft circular fiberglass tanks. After rearing for one year, each of the "family" groups are reduced to

30 fish/family (450 fish/BY) by random selection just prior to marking. All unwanted fish are returned to the supplementation production group for release in the upper Tucannon River. Fish destined for the captive broodstock program are marked by "family" group with a CWT in the snout and one in the adipose fin (backup). This is to verify "family" groups during future spawning activities so that full or half-siblings are not mated together. In addition to the CWT, an alphanumeric VI tag is placed behind the left or right eye to identify individual fish. The VI tag, should it be retained, will provide a quicker "family" identification method than the CWT. In addition, fish which retain the VI will provide individual growth rates. After the fish have been tagged, they are transferred to one of the 20 ft circular fiberglass tanks for rearing to maturity. Once the fish have been transferred to the larger rearing tanks, they are not moved again unless survival rates are greater than anticipated, or density limits are exceeded within the rearing tanks. At maturity, fish are transferred to the lower section of an adult raceway, directly below fish that have been trapped for the supplementation program. Family size and marking procedures will be the same for all BYs through the 2001 collection.

Density limits for each rearing tanks were decided prior to any stocking of fish. Most of the density limits prescribed were taken from the Dungeness River Captive Broodstock program, where same size starter and adult rearing tanks are used. Based on those density limits and expected survival and maturation rates, we were able to design the facilities needed. The current fish number maximums are as follows: 4 ft circular tanks = no more than 200 fish/tank at Age 1; 20 ft circular tanks = no more than 150 fish/tank at Age 3, or 100 fish/tank at Age 4.

#### 1997 and 1998 Brood Year Rearing

Fry from the 1997 and 1988 BYs were collected as described above, with appropriate families chosen for the program (Appendix B). Data on average length (mm), weight (g), and condition factor (K) for each "family" group are compiled during tagging (Appendix C). The actual number of parents that comprise the 1997 and 1998 BYs were 54 and 58, respectively. Mortality rates in the 1997 and 1998 BYs have remained very low. This was encouraging for the early stages of this program because facilities and rearing conditions were not ideal. For instance, the 1997 BY fish were held for an extended period of time in the 4 ft circular tanks, then had to be transferred into a sectioned off adult holding raceway. This was an extremely large rearing area for few fish. Further, uncertainties about the program's future also caused feeding schedules and rates to be interrupted frequently, which has not affected fish health, though size uniformity has been a problem for both BYs.

At the onset of FY2000 funding (October 1999), fish from the 1997 BY were starting to show signs of maturation, and 1998 BY fish had not been marked by family unit due to delays in getting funding to complete the facility modifications. Maturation and mortality rates to date for the 1997 and 1998 BYs, and marking of the 1998 BY will be discussed during the FY2000 and FY2001 activities.

## **BPA Funded Activities**

For the FY2000 funding cycle, the following items were identified within the Statement of Work to be completed for the Captive Broodstock Program:

- 1) Completion and submission of the Master Plan for the Tucannon Captive Broodstock Program by WDFW and co-managers to the Independent Scientific Review Panel (ISRP). The ISRP was then to review the Master Plan and provide recommendations for funding to the Northwest Power Planning Council (NPPC) and BPA.
- 2) Completion of National Environmental Policy Act (NEPA) by BPA, with a Finding of No Significant Impact (FONSI) prior to funds being distributed for the program.
- 3) Complete all remaining facility modification designed in the program and outlined in the Master Plan. This included completion of security fencing around the captive broodstock rearing area at LFH, a roof over the rearing tanks to provide protection, and alarm systems in place.
- 4) Provide for the care, rearing, and spawning of the captive broodstock fish on hand.
- 5) Transport all DNA samples collected since 1997 and have them analyzed to provide the genetic background of the Tucannon River spring chinook stock for future comparisons after the captive broodstock program is complete.
- 6) Continue to coordinate with co-managers, and other agencies about the progress of the Tucannon River captive broodstock program. Complete an annual report describing activities for each funded year.

# **Master Plan Development**

The Master Plan for the Tucannon River spring chinook captive broodstock program was completed in December 1999 and submitted for review and comment to the NPPC and ISRP. After review by the ISRP, comments and questions were provided to WDFW for response. WDFW responded and had two conference calls with ISRP members to discuss issues that arose during the review. After these calls and written response, the ISRP believed the program to be biologically sound and recommended that the project receive funding. The recommendation from the ISRP to the NPPC for the Tucannon Master Plan was accepted by the Council on April 4, 2000.

#### **Environmental Assessment**

Concurrent with the review of the Master Plan, WDFW assisted BPA in writing and completion of an Environmental Assessment (EA). An EA was required for the NEPA to determine that no significant impact to other species (plant or animal) would occur should the program proceed. A Finding of No Significant Impact (FONSI) was concluded after review of the EA, satisfying the

NEPA requirements before full implementation and funding of the program could occur. The FONSI was completed in May 2000, with funding available in July 2000.

# **Facility Modifications**

During FY2000, a contractor was selected for the construction of security fencing around the perimeter of the captive brood tanks. Security fencing was installed in October 2000. Outlet screens, cleaning tools, crowders, a contained-formalin treatment system, and pond covers (camouflage netting) have also been purchased for the program.

Bids were solicited for the construction of an open pole building (roof only) over the captive broodstock rearing tanks to protect the tanks from sunlight degradation, and provide additional shading for the fish. However, the open pole building could not be completed based on restrictive State engineering requirements and available funding. Funds for this portion of the project will be returned to BPA.

Hatchery staff purchased the necessary parts for the water level alarms to be installed on the captive broodstock rearing tanks. These alarms will be connected to the existing hatchery alarm system to prevent accidental loss of fish due to water supply failure.

## **Rearing and Spawning**

Captive brood fish are being reared at LFH, using standard fish culture practices and approved theraputants in pathogen free well water which is a constant 11 C. Each 20 ft circular captive tank is supplied with 150 gallons/minute (gpm), and the 4 ft tanks receive 6 gpm. To reduce the risk of catastrophic fish loss due to hatchery facility or operational failure, a number of safeguards are in place. LFH is staffed full time by personnel living on-station, providing for the protection of fish from vandalism and predation. The hatchery is also equipped with back up generators in the event of power outages. All staff are trained in proper fish handling, transport, rearing, biological sampling, and WDFW fish health maintenance procedures to minimize the risk of fish loss due to human error. All fish are handled, transported and propagated in accordance with the WDFW Fish Health Manual (WDFW 1996) and Pacific Northwest Fish Health Protection Committee (PNFHPC 1989) disease prevention and control standards to minimize loss due to disease. Sanitation procedures are employed to reduce the transfer and incidence of fish diseases and to promote quality fish in accordance with PNFHPC (1989), and Integrated Hatcheries Operations Team (1995) guidelines.

A variety of high quality commercial feed is provided through a state contract, and feed size varies with the estimated fish size of the different BYs. To date, we've used Moore-Clark Nutra<sup>TM</sup>, Moore-Clark Fry<sup>TM</sup>, and Bio-Products Salmon Brood Feed<sup>TM</sup> on the captive brood. Estimated size only is generally used to prescribe feed, as WDFW decided initially that too much handling of the fish to determine growth and size would not maintain a healthy population. This decision resulted from problems that Oregon Department of Fish and Wildlife (ODFW) and Idaho Department of Fish and Game (IDFG) captive programs had experienced during their first years of operation with monthly fish sampling (Marla Chaney, Assistant Manager, Captive

Broodstock Program, ODFW pers. comm. 2000 and Paul Kline, Principle Fisheries Research Biologist, IDFG pers. comm. 2000). All captive brood fish are reared outside under natural photo-period conditions. However, each of the 20 ft circular tanks are covered with camouflage netting which provides a shading effect over the pond. The netting also prevents fish from jumping out of the tank, and seems to maintain a "fright" response in the fish.

During the summer, captive brood fish which are Age 2 or greater are examined for signs of sexual maturation. Maturation is determined by change in body coloration and development of maturation-related morphological characteristics. Mature fish are removed and held together in an adult holding raceway typically used for summer steelhead adults at LFH. Mature fish not used for spawning are sacrificed at the end of the spawning season.

All smolts produced from the captive broodstock fish will be marked differently for identification upon adult return. Smolts will be unclipped and marked with a blank wire tag in the snout (production fish may have an adipose fin clip and CWT or VI elastomer tag and CWT). When supplementation or captive brood fish return as adults at the TFH adult trap, each unmarked (no adipose clip) adult spring chinook will be scanned for wire in the snout using a wand detector and examined for a VI tag. If the fish is not adipose fin clipped and wire is present in the snout and no VI is present, which designates the fish as likely being produced by the captive broodstock program, these fish will be passed upstream to spawn in the river. Only if the run completely collapses again, as it did in 1995, would any of the captive broodstock fish be collected for hatchery broodstock.

As stated earlier, the captive broodstock program was initiated with 1997 BY juveniles, and we have just completed selection from the 2000 BY. As of April 30, 2001, WDFW has 172 BY 1997, 262 BY 1998, 407 BY 1999, and approximately 1,190 BY 2000 fish on hand at LFH. The paragraphs below detail the selection, tagging, rearing, sorting and spawning activities, and mortalities for each BY since program inception.

1997 Brood: Following the supplementation spawning in 1997, WDFW evaluation staff used the selection criteria detailed in the Master Plan to pick family units that would be chosen for the captive broodstock population (Appendix B). However, due to a water chiller accident at LFH, many of the families originally chosen were completely destroyed. Alternate families had to be chosen for the program. Further selection also occurred because of the high incidence of deformed fish from the 1997 BY (likely a result of thermal shock from the water chiller). Even with those problems, a total of 1,200 fish were still selected for the BY. Mortality of the 1,200 fish for the 1997 BY prior to family tagging was high compared to previously documented survivals (3.3%) at LFH to the marking stage (captive brood 155 fish, 12.9%). However, this mortality rate was similar to the standard production fish over the same time period (1997 BY = 12.7%). This was likely due to the high incidence of abnormalities in the 1997 BY from the cold shock on the eggs.

Because of the uncertainty of obtaining future funding, family tagging of the 1997 BY was delayed until the 1998 BY was scheduled to come out of the incubation trays. In addition, a location had to be found to rear the 1997 BY fish after tagging. During January 2000, staff tagged a total of 433 fish with CWT and alpha-numeric VI tags for family identification. Mean length, weight, and condition factor for each family unit is described in Appendix C. Since

family units (30 fish/family) were chosen the previous September, only fish that were not showing signs of maturation were chosen. However, no records were kept on the incidence of mature fish at that time. Following tagging, fish were immediately transferred to a 10' x 100' x 8' adult steelhead holding raceway for rearing. A cover was placed over the raceway, and fish were fed with a demand feeder to minimize human disturbance.

In early October 1999, hatchery staff noted some of the 1997 BY fish were maturing at Age 2. Since no females were expected to mature, and we were awaiting completion of the 20 ft circular tanks, the fish were not sorted. It was hoped that the 20 ft circular tanks would be completed soon and immature fish would only have to be sorted once from the mature fish to move them into the captive rearing tanks. By 25 October, the tanks were finally ready to accept fish for rearing, so hatchery and evaluation staff sorted the 1997 BY. Ninety-two mature fish were removed (mature jacks) and 335 immature fish were placed into one of the 20 ft circular tanks.

During July 2000, the fish were sorted for maturity (Age 3). Mature fish (94) were removed from the 20' circular tanks and placed directly below maturing spring chinook that had been trapped from the Tucannon River as part of the supplementation program. It is theorized that maturation timing of the captive brood fish may be improved by holding the groups together (chemical pheromone influence). All remaining immature fish were split into two 20' circular tanks to reduce rearing density.

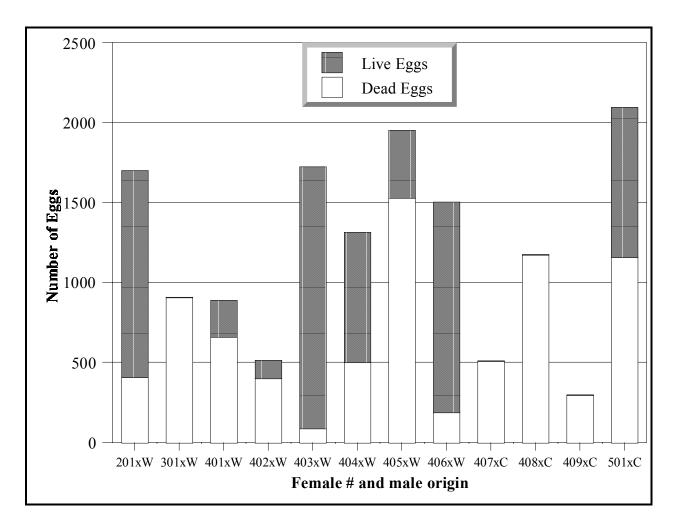
Thirteen of the 94 sorted mature fish from the 1997 BY captive broodstock were females (Appendix D). Of those, 12 were spawned (one partial spawn) and one was killed outright. The one female killed outright was not confirmed to be female until after it was killed. External sexual characteristics were not obvious (except coloration) and it was therefore difficult to tell sex until after it was dead. This fish was very small, and estimated fecundity was less than 300 eggs.

Captive brood females spawned over a four week period, with peak eggtake (9 females) on 19 September. This was about one week off the peak spawning date for the supplementation program, and for naturally spawning fish in the Tucannon River (Bumgarner et al, 2000). Total collection of eggs that were fertilized was 14,577 eggs. Mean fecundity based on the 11 fully spawned females was 1,298 eggs/female and initial egg survival was 47.3%. High egg mortality was most likely related to age of spawners (Age 3) and was expected for three-year-old captive brood females (Dan Witczak, Fish Hatchery Specialist 3, WDFW Hurd Creek Hatchery, Dungeness River spring chinook captive broodstock program, pers. comm., 2000). Following spawning in 2000, production of three-year-old females was immediately identified as not a goal for the Tucannon captive broodstock program. However, because of accelerated growth in the 1998 and 1999 BYs they are expected in the future. Recent size recommendations for the program at each age class will hopefully decrease the degree of early maturation. Program emphasis in the future is to produce Age 4 and Age 5 fish (and potentially Age 6), where egg survival is expected to be higher.

Mean egg size (g/egg) from the captive broodstock was smaller than Age 5 wild and hatchery origin fish (Table 1), but were similar in size to Age 4 natural and hatchery fish from the supplementation program. Eight of the 12 spawned females were crossed with wild (unmarked)

males from the supplementation program, with the remaining four crossed with males from the 1997 captive brood (Figure 5). Male origin did not appear to influence egg survival. Spawn timing for the captive broodstock females was about one week later than observed for the supplementation fish. It is unknown whether holding mature captive fish with the supplementation fish, constant rearing fish under natural photo-period light, constant well water temperature, or all of them, had influenced spawn timing. Future testing may be conducted to evaluate the hypothesis.

Table 1. Comparison of mean length and mean egg size by spawner origin and age.										
Spawner Origin and Age	Mean Fork Length (cm)	SD	Mean Egg Size (g/egg)	Range						
Captive Brood (Age 3)	48.3	5.5	0.20	0.16-0.27						
Wild Origin (Age 4)	68.3	3.8	0.21	0.15-0.33						
Hatchery Origin (Age 4)	69.6	4.0	0.25	0.10-0.32						
Wild Origin (Age 5)	84.5	3.4	0.27	0.13-0.35						
Hatchery Origin (Age 5)	77.5	4.1	0.27	0.19-0.34						



**Figure 5.** Fecundity and crosses of the 1997 brood year captive broodstock spawned in 2000.

As of 30 April, 2001, WDFW has 4,211 captive broodstock progeny on hand. These fish will be tagged only with a blank wire tag in the snout in September 2001. They will be released as smolts into the Tucannon River during March/April 2002 along with the supplementation fish from Curl Lake AP. A sub-sample of the captive brood progeny and supplementation fish will be Passive Integrated Transponder (PIT) tagged prior to release from Curl Lake AP to monitor their downstream migration.

Fish from the 1997 BY have remained relatively healthy throughout their rearing at LFH, with 37 mortalities (8.5% of the original tagged population) between January 1999 and April 2001 not related to maturation (Figure 6). Most of the immature mortalities occurred following the transfer of fish into the 20 ft circular tanks in October 1999. The mortalities likely resulted from handling and lower doses of formalin which were ineffective in controlling external fungus. Stronger formalin dosages/treatments were applied and the mortalities ceased.

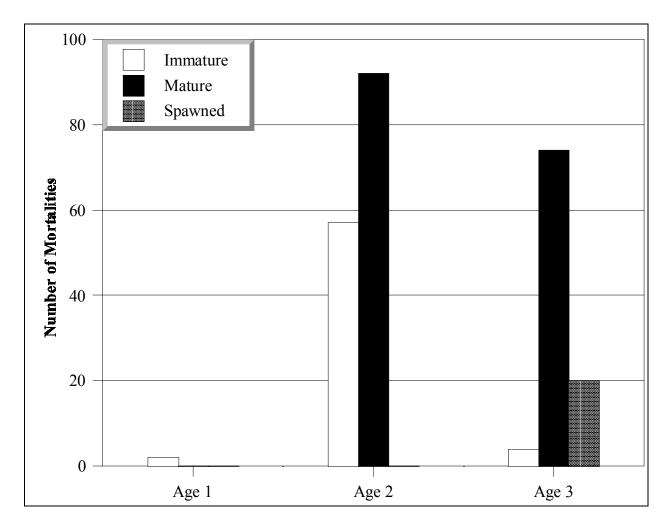


Figure 6. Number of mortalities by age for each stage of maturity for the 1997 brood year.

**1998 Brood:** Following the supplementation spawning in 1998, evaluation staff used the selection criteria detailed in the Master Plan to pick family units for the captive broodstock

population (Appendix B). Twelve hundred fish were selected for the 1998 BY. Mortality of the 1998 BY prior to family tagging remained low (21 fish, 1.8%). This was a similar mortality rate as documented for the standard production fish over the same time period (1998 BY = 3.8%) from December 1998 to October 1999.

During October of 1999, staff tagged 438 fish with CWT and alpha-numeric VI tags for family identification. Mean length, weight, and condition factor for each family unit is provided in Appendix C. A total of 12 fish were not tagged as they exhibited external signs of maturation. Due to delays in construction of the 20 ft circular tanks, fish were tagged and held inside in a rearing trough and allowed to heal for about one month. After the first day of tagging, eight fish were lost from jumping out of the rearing trough. Additional netting was installed over the trough, and no additional mortalities occurred. Fish were transferred from the rearing trough in November 1999 to one of the 20 ft circular tanks.

Since the 1998 BY was the second BY in production, and would be Age 2 in September 2000, we were confident that all maturing fish would be males. We also felt that few of the 1997 BY would be maturing females. Since adequate males would likely be represented in the spawning population from the 1997 BY, we decided that any mature males from the 1998 BY would be killed and not used during spawning in 2000. Cryopreservation was identified as an option for the maturing males (WDFW 1999); however, this option was identified as a low priority for this program because of the large historic repository of natural origin males.

Since all maturing 1998 BY males were to be killed, sorting was delayed until mid-September 2000. In total, we sorted 142 (32.4% of the starting population) mature males from the 1998 BY. Immature fish from the 1998 BY were not split between circular tanks as rearing densities are expected to remain below allowable maximum until the fish are past Age 3.

Fish from the 1998 BY have remained healthy throughout their rearing at LFH, with only 17 mortalities to date not related to maturation (Appendix D).

<u>1999 Brood</u>: WDFW started FY2000 just after the spawning of the supplementation fish was complete in 1999. In October and November 1999, origin, crosses, and ELISA results were evaluated to choose the 15 family units. During December, 1,200 fish from the 1999 BY representing 15 family units were selected (Appendix B) and placed within the captive broodstock inclosure in the 4 ft rearing tanks. Mortality of the 1999 BY prior to family tagging remained low (20 fish, 1.7%). This is a similar mortality rate documented for the standard production fish over the same time period (1999 BY = 1.3%) from November 1999 to October 2000.

During September 2000, family sizes were reduced to about 30 fish/family. On 5 October, these fish were tagged with a CWT and an alpha-numeric tag behind the eye, and then placed in a single 20 ft circular rearing tank. Mean length, weight and condition factor for each family unit is provided in Appendix C. Total number of fish tagged to remain in the captive broodstock population was 409 fish. Forty-three precocial males were removed and not tagged during the tagging operations. The number of precocial males observed for the 1999 BY is likely a factor of the larger size fish at Age 1. For the future two BYs remaining, we will reduce daily feeding

rates to produce fish of a smaller size at Age 1, as was achieved for the 1997 and 1998 BYs. Since tagging of the family groups, mortality has remained very low (Appendix D).

**2000 Brood:** In late August through September 2000, WDFW spawned fish from the supplementation program at LFH. During October 2000, priority selection of the 2000 BY fish (Appendix B) was based on crosses (WxW, HxW, HxH), origin, and ELISA results according to the Master Plan. Eighty fish from each of the 15 family units selected (1,200 fish total) were moved outside to the 4 ft circular rearing tanks located in the captive broodstock enclosure in December 2000.

#### **DNA Genetic Samples**

At the beginning of the program in 1997, evaluation staff collected DNA samples from all spring chinook parents that eventually contributed gametes to the captive broodstock population. Additional samples were also collected from other Tucannon River origin spring chinook carcasses to provide a large genetic data set that will describe the population. All 1997-1999 fin clip and opercle punch DNA samples were consolidated from Tucannon River spring chinook adults and shipped to WDFW's genetics lab. Evaluation staff coordinated which samples were the highest priority for the captive broodstock program. These samples will be analyzed in Summer, 2001. Results from the tests were not available at the completion of this report, but will be reported at a future date.

Opercle punches for DNA analysis were also collected from all 2000 spawners, including captive brood spawners from the 1997 BY (three-year-olds). All 2000 DNA samples were sent to the WDFW genetics lab in Olympia for baseline DNA analysis.

# **Coordination and Reporting**

Ever since BPA funding was acquired, WDFW has joined other researchers in a group known as the Captive Broodstock Technical Oversite Committee (CBTOC). This CBTOC committee brings together all BPA funding projects working with captive broodstock or captive rearing programs to make sure that all groups are coordinated and communication is occurring between projects. The CBTOC also gives each of the researchers a chance to ask questions about other programs success and failures, so each respective program can be adapted for the better.

In addition, WDFW formed its own Technical Working Group (TWG) which consists of WDFW project personnel, and representatives from the NPT and CTUIR. The group was formed so WDFW and co-managers could make unified decisions regarding questions about the Tucannon Spring Chinook captive broodstock program. To date, this group has had one meeting, which was more informational to the NPT and CTUIR, than used for decisions. Future meetings will be held to inform the co-managers of the progress to date, and to resolve any issues that arise.

To satisfy the Section 10 permit requirements, WDFW also provides NMFS with a monthly update on the captive broodstock and supplementation program activities. This monthly program update also provides to the co-managers monthly inventory of fish on hand, mortalities incurred, and any actions coming up (i.e., sorting of mature fish) that may warrant their attention.

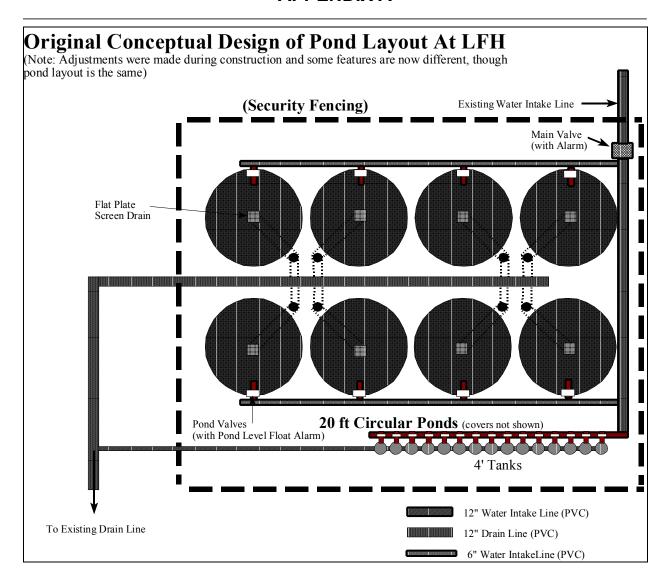
This annual progress report will be the first in a series of annual progress reports produced by WDFW to monitor and disseminate the information gathered from this project to other researchers in the Columbia and Snake river basins. Additional report and papers will also be published in the future following complete returns of all captive brood origin fish back to the Tucannon River.

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#### **APPENDIX A**



# **APPENDIX B**

Table 1. Selection of progeny for the Tucannon River spring chinook captive broodstock program based on origin, crosses and BKD ELISA results, 1997 and 1998 BYs.											
origin, ci		<b>BKD ELISA results,</b> Female Numbers	1997 and 1998 BYs.  Male Numbers	Crosses	BKD ELISA	Tank/Family					
Year	Eggtake Date	remaie Numbers	Maie Numbers	Closses	BKD ELISA	Number					
97	09/16	H885 + H886	W108+W110	Mixed	LOW, BL	TANK 1					
97	09/16	H889	W116-W120	Mixed	BL	TANK 2					
97	09/23	W958 + W957	H122+H123	Mixed	BL	TANK 3					
97	09/16	W897 + W898	H156-H199	Mixed	BL	TANK 4					
97	09/09	H872 + H871	W159-W161	Mixed	BL	TANK 5					
97	09/09	H873	W163+W165	Mixed	LOW	TANK 6					
97	09/09	W881 + W882	H167+H175	Mixed	BL	TANK 7					
97	09/16	W951 + W952	H149+H157	Mixed	BL	TANK 8					
97	09/09	W874 + W875	H171+H173	Mixed	BL	TANK 9					
97	09/09	W878 + W876	H179+H181	Mixed	LOW, BL	TANK 10					
97	09/02	W869 + W867	H191+H193	Mixed	BL	TANK 11					
97	09/09	H879	W169+W177	Mixed	BL	TANK 12					
97	09/16	W899	H153+H154	Mixed	BL	TANK 13					
97	09/02	W870	H183+H185	Mixed	BL	TANK 14					
97	09/02	H868	W187+W189	Mixed	BL	TANK 15					
98	08/25	W1003 + W1004	H754-H753	Mixed	BL	TANK 1					
98	08/25	W1005 + W1006	H751-W131	Mixed	LOW, BL	TANK 2					
98	09/08	W3001 + W3002	H758-H759	Mixed	LOW, BL	TANK 3					
98	09/08	W3003 + W3004	H755-H756	Mixed	BL	TANK 4					
98	09/08	W3005 + W3006	H757-H760	Mixed	BL	TANK 5					
98	09/08	W3007 + W3008	W128-W129	Mixed	BL	TANK 6					
98	09/08	H3009 + H3010	W130-W133	Mixed	LOW, BL	TANK 7					
98	09/11	H4001 + H4002	W135-W134	Mixed	LOW, BL	TANK 8					
98	09/11	W4003 + W4004	H762-H761	Mixed	LOW, BL	TANK 9					
98	09/11	W4007 + W4008	H767-H765	Mixed	LOW, BL	TANK 10					
98	09/11	W4009 + W4010	H769-H768	Mixed	BL	TANK 11					
98	09/15	W5002	H777-H773	Mixed	LOW	TANK 12					
98	09/15	W5003	H772-H771	Mixed	LOW	TANK 13					
98	09/22	W6005 + W6006	H781-H780	Mixed	BL	TANK 14					
98	09/22	W6007 + W6008	H783-H782	Mixed	BL	TANK 15					

Table 2. Selection of progeny for the Tucannon River spring chinook captive broodstock program based on origin, crosses and BKD ELISA results, 1999 and 2000 BYs.											
99	08/31	H101	H1+H2+H526	Hatchery	LOW	TANK 1					
99	09/07	H203	H12+H13+H536	Hatchery	BL	TANK 2					
99	09/07	H204	H15+H530+H531	Hatchery	LOW	TANK 3					
99	09/07	W205	H18+H532+H533	Mixed	LOW	TANK 4					
99	09/07	H206	H528+H529+H534	Hatchery	BL	TANK 5					
99	09/07	H212	H19+H20	Hatchery	BL	TANK 6					
99	09/14	H305	W31+H571	Mixed	LOW	TANK 7					
99	09/14	H306	W21+H576	Mixed	LOW	TANK 8					
99	09/14	H307	H40+H550	Hatchery	LOW	TANK 9					
99	09/14	H309	H23+H549	Hatchery	BL	TANK 10					
99	09/14	H310	H39+H572	Hatchery	LOW	TANK 11					
99	09/14	H311	H36+H568	Hatchery	LOW	TANK 12					
99	09/14	H312	H24+H544	Hatchery	LOW	TANK 13					
99	09/21	H403	H45+H580	Hatchery	LOW	TANK 14					
99	09/21	H404	H581+H582+H583	Hatchery	LOW	TANK 15					
00	8/29	H102	H1+H2	Hatchery	BL	TANK 1					
00	8/29	H103+H104	H3+H4	Hatchery	BL	TANK 2					
00	8/29	H105+W106	H5+H6	Mixed	BL	TANK 3					
00	9/05	H202	W1+H19	Mixed	BL	TANK 4					
00	9/05	H203+H204	W2+H7	Mixed	BL	TANK 5					
00	9/05	H205+H206	H8+H9	Hatchery	BL	TANK 6					
00	9/05	H209+H210	H12+H13	Hatchery	BL	TANK 7					
00	9/05	H211	H14+H15	Hatchery	BL	TANK 8					
00	9/05	H213+H214	H16+H17	Hatchery	BL	TANK 9					
00	9/05	W215	H10+H11	Mixed	BL	TANK 10					
00	9/12	H301+H302	H20+H24	Hatchery	BL	TANK 11					
00	9/12	H303+H304	W3+H23	Mixed	BL	TANK 12					
00	9/12	H308+H311	W5+H22	Mixed	BL	TANK 13					
00	9/19	W401+H402	H30+H31	Mixed	BL	TANK 14					
00	9/19	H403+H404	W6+H32	Mixed	BL	TANK 15					

# **APPENDIX C**

Brood	Family	Number of	Mean Length		Mean Weight		
Year	Unit	Fish	J	S.D.	Z.	S.D.	K
1997	1	29	113	7.8	19.4	4.4	1.31
1997	2	14	110	5.2	17.3	2.7	1.29
1997	3	31	125	9.1	28.4	6.0	1.44
1997	4	29	118	9.3	22.7	6.0	1.37
1997	5	31	119	9.3	22.7	5.8	1.30
1997	6	30	119	8.6	22.6	5.2	1.33
1997	7	30	117	7.2	21.3	4.3	1.32
1997	8	29	121	10.2	24.8	6.8	1.36
1997	9	30	117	8.1	21.8	5.0	1.32
1997	10	30	115	11.0	19.7	6.1	1.27
1997	11	30	101	6.4	13.1	2.6	1.25
1997	12	30	120	12.5	24.5	8.0	1.38
1997	13	30	121	9.3	24.4	6.6	1.34
1997	14	30	112	6.2	18.8	3.2	1.33
1997	15	30	109	9.6	18.7	4.8	1.41
	/ Means	433	116	10.5	21.5	6.4	1.34
Totals	/ Ivicalis	433	110	10.5	21.3	0.4	1.54
1998	1	20	120	15.6	22.2	8.6	1.23
1998	1 2	30 29	108		22.3 15.9		
1998		30		10.0		5.0	1.25
	3		112	13.1	18.6	7.8	1.26
1998	4	30	112	11.5	17.7	6.4	1.24
1998	5	30	117	16.0	20.5	9.9	1.20
1998	6	28	117	15.0	21.6	11.0	1.26
1998	7	32	120	18.0	23.2	11.6	1.26
1998	8	30	129	12.0	26.5	7.8	1.21
1998	9	30	121	16.9	23.0	9.9	1.24
1998	10	28	130	9.0	26.0	4.9	1.18
1998	11	25	120	13.6	22.3	7.7	1.26
1998	12	31	127	10.1	24.0	4.9	1.16
1998	13	29	122	11.4	22.0	6.7	1.19
1998	14	27	120	13.2	21.6	7.7	1.20
1998	15	29	138	11.0	30.3	6.7	1.14
Totals	/ Means	438	121	15.2	22.4	8.7	1.22
1999	1	27	147	14.6	41.1	11.3	1.25
1999	2	28	138	13.1	35.7	8.9	1.34
1999	3	28	133	11.6	33.9	11.3	1.42
1999	4	30	145	8.9	39.2	6.7	1.27
1999	5	25	136	15.8	35.4	11.8	1.34
1999	6	30	136	10.7	33.8	8.9	1.32
1999	7	27	129	20.9	30.0	14.8	1.29
1999	8	29	129	12.0	29.9	9.0	1.35
1999	9	25	128	16.3	29.3	11.6	1.33
1999	10	23	130	18.9	31.0	14.4	1.32
1999	11	23	137	13.1	36.0	10.7	1.37
1999	12	28	141	13.5	38.4	10.2	1.33
1999	13	30	133	13.9	31.9	9.1	1.34
1999	14	30	133	10.7	31.6	7.6	1.32
1999	15	26	132	16.6	34.1	14.1	1.39
	/ Means	409	135	15.1	34.1	11.2	1.33

### **APPENDIX D**

Table 1. Tucannon River spring chinook captive broodstock mortalities by family unit, sex, age, and maturity for the 1997 Brood

		Males								emales				
		Age 1	Ag	e 2		Age 3		Age 1	Age 2		Age 3			
Family	Starting												Total	Percent
Unit	Population	IM	IM	MA	IM	MA	SP	IM	IM	IM	MA	SP	Mortality*	Mortality
1	29		1	4		6			3				14	48
2	14			4		1	2						8	57
3	31		3	4		3		1					12	39
4	29		2	4		10			3			1	20	69
5	31			8		7	1		4	1			21	68
6	30		2	13		1			3	1	1		21	70
7	30		1	5		5	1		3				15	50
8	29			14		1			1			2	18	62
9	30		2	6		5	2		4				19	63
10	30		1	7		5			3			3	19	63
11	30	1	2	3		6	1		3				16	53
12	30		2	5		4			3			4	18	60
13	30		1	7		4			1	1			15	50
14	30		1	1		13	1			1		1	18	60
15	30		1	7		2			7			1_	18	60
Totals	433	1	19	92	0	73	8	1	38	4	1	12	252	58

IM=Immature, MA=Mature, SP=Spawned \*Totals include 3 fish of unknown sex.

Table 2.	Tucannon River spring chinoo	k captive broodstock mortal	ities by family unit, sex, a	age, and maturity for the 1998 Brood	
Year					

i cai.														
		Males							- I	Females				
		Age 1	Ag	e 2		Age 3		Age 1	Age 2		Age 3			
Family	Starting												Total	Percent
Unit	Population	IM	IM	MA	IM	MA	SP	IM	IM	IM	MA	SP	Mortality*	Mortality
1	30			12									12	40
2	29			9									10	35
3	30			11				1	1				15	50
4	30		1	10					1				12	40
5	30			8					1				9	30
6	28		2	5									7	25
7	32			8									8	25
8	30		1	9									11	37
9	30			5									6	20
10	28			15									16	57
11	25			10									10	40
12	31	1		11				1					13	42
13	29			8									8	28
14	27			10									10	37
15	29	3		11				4					20	69
Totals	438	4	4	142				6	3				167	38

IM=Immature, MA=Mature, SP=Spawned

<sup>\*</sup>Totals include 8 fish of unknown sex.